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This project uses single crystal x-ray diffraction methods for the detailed study of the interactions between effector molecules and their receptors. A small soluble protein from bovine nasal epithelium that binds a variety of odorants (OBP) will be the subject of this study. Crystals of OBP were obtained in our laboratory that contain 2 OBP dimers in the asymmetric unit. The structure is being solved using isomorphous heavy-atom derivatives and the maps will be enhanced by density averaging and map inversion. The polypeptide chain will be traced using the available OBP sequences (rat and bovine). The structures of several odorants bound to OBP will be determined by difference Fourier methods to understand the characteristics of the initial events in olfactory perception and the nature of chemical discrimination in smell. These studies will provide a unique insight for understanding the functioning of OBP and for answering important questions about receptor recognition in general.

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**Specificity of Odor Recognition:
The Three-Dimensional Structure
of an Odorant Binding Protein**

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ONR PROGRESS REPORT

OBJECTIVE: The long range objective of this project is to study the structural basis of the specificity and affinity of biological receptors. As part of this investigation we are determining the three dimensional structure of an odor Binding Protein (OBP) from the bovine nasal olfactory epithelium, using single-crystal x-ray diffraction techniques. In addition, we are using the same methods for studying the structures of complexes of OBP with several odorants.

PROGRESS (Year 1)

Purification and Crystallization We have improved the method for the preparation of OBP by adding an hydrophobic interaction chromatography step using HPLC. We have prepared large amounts of this highly purified OBP and set up extensive crystallization experiments. Large crystals are now routinely obtained by vapor diffusion using macro-seeding techniques.

Data Collection and Structure Analysis We had initially collected x-ray data sets of the native and a heavy atom derivative on a four-circle diffractometer. We collected a 5A resolution data set for the native OBP and a 6A resolution data set of heavy atom derivative p-chloro mercuric Benzyl sulphonic acid (PCMBS). The mean fractional isomorphous difference between the native and heavy-atom derivative is 13.7%. These data sets have been processed and used for a) Self Rotation function of the native data and b) difference Patterson analysis with native and derivative data.

The Rotation function analysis has yielded a pseudodiad (local axis) which is independent of the three crystallographic two-fold screw-axes. This confirms our assumption of two molecules in the asymmetric unit of this crystal form. Furthermore, the ΔF (difference between native and derivative structure amplitudes) Self Rotation function analysis is in agreement with the same two-fold non-crystallographic axis. The location of this axis is at $\psi=90$, $\phi=30$ at $\kappa=180$.

Using the Area detector at the National Bureau of Standards, (kindly authorized by Alex Wlodawer), we collected a native OBP data to 3.3A resolution and a heavy-atom derivative (K_2PtCl_4 to 3.5A) using a sealed tube generator. Since the installation of the Nicolet Area Detector with a rotating anode source at our site, we have recollected the same data sets to 3.0A and 3.2A resolution respectively. Additionally, we have also recollected data sets for the PCMBS derivative to 3.8A.

Table I - Summary of Area Detector Data

	Rsym(%)	Mean, fractional, isomorphous, difference (%)
3.0A Native Data Set	7.8%	N/A
3.3A Native Data set	5-13%	N/A
3.5A K ₂ PtCl ₄ heavy atom derivative	6.93%	18.7%
3.8A PCMBS derivative	7.8%	23.4%
3.2A K ₂ PtCl ₄ derivative	(data is being collected and processed at the moment).	

We have been following three strategies for structure solution. Firstly, by molecular replacement using the coordinates of Insectocyanin (coordinates kindly provided by Dr. Ivan Rayment & Dr. Hazel Holden), secondly, using the single isomorphous replacement derivative and the solvent flattening technique proposed by B.C. Wang and thirdly, the Multiple Isomorphous replacement technique.

The results so far with molecular replacement with Insectocyanin did not look hopeful. Hence, we are proceeding with the methods that use heavy-atom derivatives. The density modification method has been useful in defining the molecular boundary (dimer) at 5A resolution. We have also taken advantage of the local two-fold axis derived from the self-rotation function, and performed density averaging for the two molecules in the asymmetric unit.

PROPOSED WORK (Year 2)

Map Calculation We will use the new native and derivative data sets to calculate the difference Patterson function of the K₂PtCl₄ derivative to 3.2A and PCMBS to 3.8A. These functions will be used to obtain the atomic coordinates of the heavy-atoms in the unit cell. The heavy-atom parameters will be refined and the native phases calculated with both derivatives will be used to compute an electron density map of the crystal. If necessary the electron density map will be improved using solvent flattening and density averaging around the local two-fold axis.

Map Interpretation We now have access to the sequence of the first 160 amino-acids of the OBP (Hugo Monaco, personal communication) and the rest of the sequence will become available shortly. We will attempt to trace the polypeptide chain of both monomers of the OBP in the electron density making use of the available sequence information. OBP was found to have sequence similarities with other proteins that also bind hydrophobic molecules. Three of these proteins -Retinol binding Protein (RBP), β -lactoglobulin and Insecto-cyanin- have known three-dimensional structures. In tracing the polypeptide chain of OBP we will make extensive use of the published structures of these three proteins. This information will facilitate significantly the interpretation of the electron density map.

Crystals of OBP-odorant complexes We have shown that OBP crystals can be prepared in the presence of several odorants (dimethyloctanol, several pyrazines, amylacetate). The crystals are isomorphous even at odorant concentrations much higher than the odorants' dissociation constants suggesting that they contain OBP-odorant complexes. We plan to prepare OBP crystals each substituted with one of several odorants and we will collect three-dimensional X-ray diffraction data using our area detector system. Fourier maps will be calculated using native phases and the differences between substituted and native structure-factor amplitudes as coefficients. The electron density obtained in these maps will be superimposed on the OBP model and the interactions between the different odorants and the protein will be characterized in detail.

The identification of the residues involved in binding different odorants and the chemical nature of the interactions will be used to address important questions about the activity of the OBP. What is the extent of overlap between the binding site for different odorants? Do all odorants bind to the same site in OBP? Do all the odorants interact with the same or with different OBP residues? Are residues in the combining site of OBP used for the same function for different odors (for example, a given serine side chain is always used to bind H-bond acceptors; the same phenyl alanine side chain is commonly used to bind aromatic rings, etc.)?